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Utility of Mycobacterial Interspersed Repetitive Unit Typing for Differentiating Multidrug-Resistant *Mycobacterium tuberculosis* Isolates of the Beijing Family

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Mycobacterial interspersed repetitive unit (MIRU) typing has been found to allow rapid, reliable, high-throughput genotyping of *Mycobacterium tuberculosis* and may represent a feasible approach to study global *M. tuberculosis* molecular epidemiology. To evaluate the use of MIRU typing in discriminating drug-resistant *M. tuberculosis* strains of the Beijing genotype family, 102 multidrug-resistant (MDR) clinical isolates and 253 randomly selected non-MDR isolates collected from 2000 to 2003 in Hong Kong were subjected to 12-locus MIRU typing, spoligotyping, and IS*6110* restriction fragment length polymorphism (RFLP) typing. Spoligotyping showed that 243 (68.5%) of 355 isolates belonged to Beijing family genotype. MIRU typing showed lower discrimination in differentiating between the Beijing family strains (Hunter-Gaston discriminative index [HGI] of 0.8827) compared with the IS*6110* RFLP method (HGI = 0.9979). For non-Beijing strains, MIRU typing provided discrimination (HGI = 0.9929) comparable to that of the RFLP method (HGI = 0.9961). There was no remarkable difference in discrimination power between the two methods in differentiating both within and between MDR and non-MDR strains of *M. tuberculosis*. Dendrograms constructed with the MIRU typing data showed a clear segregation between the Beijing and non-Beijing genotype. Addition of RFLP to MIRU typing offered a higher discrimination ability (92.6%) than did addition of MIRU typing to RFLP (40.0%). This supported the potential use of this method to analyze the global genetic diversity of MDR *M. tuberculosis* strains that may be at different levels of evolutionary divergence.

Tuberculosis (TB) remains a major public health threat worldwide, and an estimated one-third of the world population has latent infection (6). Outbreaks of multidrug-resistant (MDR)-TB and exponential development of international travel have further extended this threat (9, 11). In several countries in Asia where TB incidence is high, a specific family of *Mycobacterium tuberculosis* known as the Beijing genotype was found to be highly prevalent (29). During the last decade, Beijing genotype has been successful in spreading in different geographic locations (2, 17, 19). Possible associations with drug resistance (3, 5) and high adaptability to the host intracellular environment (20) have been reported.

Restriction fragment length polymorphism (RFLP) analysis based on the insertion sequence IS*6110* is a powerful tool for studying the molecular epidemiology and is considered a "gold standard" for DNA fingerprinting of *M. tuberculosis*. The procedures involved, however, are time-consuming and technically demanding, as well as requiring large quantities of DNA. Moreover, Beijing family strains often carry more than 20 copies of IS*6110* in their genome, with a high degree of similarity in band pattern, which makes computer-assisted analysis particularly difficult and cumbersome. This is especially relevant for laboratories in countries with a high burden of infection, where resources are often limited and consistent RFLP testing may not be feasible, making interlaboratory comparisons especially difficult. Ideally, a typing method should be technically simple and the results should be easily displayed digitally for comparison across laboratories.

Due to the genetic homogeneity of the Beijing family, spoligotyping cannot be used for the differentiation of Beijing genotype strains (29), although spoligotyping possesses most of the features of an ideal typing method. A PCR-based typing method using variable number tandem repeats (VNTRs) of genetic elements, mycobacterial interspersed repetitive units (MIRUs) in 12 human minisatellite-like regions of the *M. tuberculosis* genome, has been developed and has produced some promising initial results (16, 24, 25). Studies have shown that the discriminatory power of this method may be comparable to that of IS*6110* RFLP. Moreover, MIRU typing also showed its usefulness in studying the population structure of *M. tuberculosis* (22, 26). These studies, however, included no or very few Beijing family strains.

In developing countries with limited resources, it may not be feasible to type all *M. tuberculosis* isolates. In such countries, when setting the priority on which set of strains to type in order of importance to national TB control programs, typing of drug-resistant isolates definitely has high priority because of the direct impact of these isolates on the effectiveness of chemotherapy. The aim of the present study was to investigate the

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TABLE 1. Primer sequence and repeat unit size of the MIRU loci in this study

MIRU locus	PCR primer sequence (5′-3′)	Predicted size + additional unit (bp)	Amt (pmol) in one PCR tube
MIRU-2	TGGACTTGCAGCAATGGACCAACT TACTCGGACGCCGGCTCAAAAT	402 + 53	4
MIRU-4	GTCAAACAGGTCACAACGAGAGGAA CCTCCACAATCAACACACTGGTCAT	114 + 77	4
MIRU-10	GTTCTTGACCAACTGCAGTCGTCC GCCACCTTGGTGATCAGCTACCT	482 + 53	4
MIRU-16	TCGGTGATCGGGTCCAGTCCAAGTA CCCGTCGTGCAGCCCTGGTAC	565 + 53	6
MIRU-20	TCGGAGAGATGCCCTTCGAGTTAG GGAGACCGCGACCAGGTACTTGTA	437 + 77	2
MIRU-23	CTGTCGATGGCCGCAACAAAACG AGCTCAACGGGTTCGCCCTTTTGTC	150 + 53	4
MIRU-24	CGACCAAGATGTGCAGGAATACAT GGGCGAGTTGAGCTCACAGAA	395 + 54	4
MIRU-26	TAGGTCTACCGTCGAAATCTGTGAC CATAGGCGACCAGGCGAATAG	285 + 51	4
MIRU-27	TCGAAAGCCTCTGCGTGCCAGTAA GCGATGTGAGCGTGCCACTCAA	498 + 53	4
MIRU-31	ACTGATTGGCTTCATACGGCTTTA GTGCCGACGTGGTCTTCAT	492 + 53	2
MIRU-39	CGCATCGACAAACTGGAGCCAAAC CGGAAACGTCTACGCCCCACACAT	540 + 53	4
MIRU-40	GGGTTGCTGGATGACAACGTGT GGGTGATCTCGGCGAAATCAGATA	354 + 54	2

differentiation ability of MIRU typing of MDR and non-MDR *M. tuberculosis* isolates of Beijing genotype and other strains. This will help assess the feasibility of using this method in TB epidemiological study in areas where Beijing genotype strains are prevalent.

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MATERIALS AND METHODS

Bacterial strains. Three hundred eighty nonduplicate drug-resistant clinical strains of *M. tuberculosis*, obtained from 2000 to 2003, in the Hong Kong TB Reference Laboratory were selected for this study. Each strain came from a different patient. These strains included all MDR strains (110 strains) and 270 randomly selected non-MDR strains from a total of 814 obtained from clinical specimens during the study period. Most of these strains showed either monoresistance to streptomycin or isoniazid or combined resistance to these two drugs. These organisms were recovered from the −70°C stock which was stored in Trypicase soy broth with glycerol and were grown on Löwenstein-Jensen medium for 3 weeks at 37°C. The recovery rate was over 95%. Mycobacterial identification and testing of the drug susceptibility of these strains to first-line anti-TB drugs were performed as previously described (13). MDR was defined as resistance to at least isoniazid and rifampin.

Molecular typing methods. IS*6110* RFLP and spoligotyping of the *M. tuberculosis* strains were performed by standardized methods (18, 19). Briefly, genomic DNA was extracted and digested with PvuII and subjected to agarose gel electrophoresis. After DNA was blotted to a Hybond membrane, DNA fingerprinting was performed by hybridization with the IS*6110* insertion sequence using the enhanced chemiluminescence (Amersham) (28). Spoligotyping detected the presence of 43 spacer sequences in the direct- repeat region by reversed-line blot hybridization (14). The Beijing family genotype was identified by its unique spoligotyping pattern, in which only 9 of the 43 spacers sequence were present (29).

MIRU typing was performed by the method of Frothingham and Meeker-O'Connell (7). Briefly, bacteria were first suspended in 200 μl of milli-Q water, boiled for 10 min, and cooled in an ice bath immediately for 5 min. A 2-μl volume of this lysed bacterial supernatant was added to the PCR mixture of each MIRU-PCR reaction mix to a final volume of 20 μl; this mixture contains 0.1 μl of HotStartTaq DNA polymerase (0.5 U) (Qiagen) with 4 μl of Q-solution, 0.5 mM each dATP, dCTP, dGTP, and dTTP, 2 μl of PCR buffer, variable concentrations of each primer, and 1.5 mM $MgCl_2$ in 1X PCR buffer. The oligonucleotides used (Table 1) in the PCR corresponded to flanking regions of the polymorphic MIRU loci identified in the H37Rv genome (25). PCRs were run in DNA thermal cyclers (model 9700; Perkin-Elmer) under the following conditions: 95°C for 15 min, followed by 40 cycles of 94°C for 1 min, 59°C for 1.5 min, and 72°C for 1.5 min, with a final extension at 72°C for 10 min. PCR products were analyzed on a 2% NuSieve agarose gel (Applied Biosystems). The allele-naming table (Table 1) was prepared by the method of Frothingham and Meeker-O'Connell (7).

Computer-assisted and statistical analysis. BioNumerics software (v 3.0; Applied Maths) was used to analyze molecular typing results. IS*6110* RFLP patterns were analyzed as fingerprint types, while MIRU types were analyzed as character types. Similarities between MIRU types were calculated by the categorical coefficient in which all MIRU loci were weighted equally. This procedure counted the number of matched loci between pairs of isolates; when there was a difference, they were scored as unmatched irrespective of the number of repeats present (thus, 1 versus 5 scored the same, i.e., unmatched, as 1 versus 2). A dendrogram was constructed by the unpaired group method using arithmetic averages. The level of discrimination of each typing method was calculated by using the Hunter-Gaston discriminatory index (HGI) (12).

RESULTS

Spoligotyping and Beijing family genotype. A total of 355 *M. tuberculosis* strains were recovered for this study, including 102 MDR-TB strains (28.7%) and 253 non-MDR-TB strains (71.3%) (Table 2). Spoligotyping, by examining the deletion of spacers 1 to 34, identified 243 *M. tuberculosis* isolates (68.5%) to be members of the Beijing family genotype. For the 112 non-Beijing isolates, the IS*6110* RFLP study showed 88 isolates as having five or more IS*6110* bands and 24 with fewer than five bands. The proportions of Beijing genotype among MDR and non-MDR isolates were found to be similar, with 72 (70.6%) of MDR-TB strains and 171 (67.6%) of non-MDR strains belonging to the Beijing genotype.

MIRU typing. All *M. tuberculosis* isolates were subjected to analysis of 12 MIRU loci (Table 1). MIRU typing detected a

TABLE 2. Comparison of the discriminatory power of various genetic markers between the Beijing and non-Beijing genotypes

Discriminatory parameter	Beijing genotype (n = 243)	Non-Beijing genotype[a]			All strains (n = 355)
		All strains (n = 112)	>5 bands (n = 88)	<5 bands (n = 24)	
MIRU					
Total type pattern	60	83	68	17	135
No. (%) of isolates having unique type	32 (13.2%)	65 (58.0%)	54 (61.4%)	14 (58.3%)	91 (25.6%)
No. of clusters	27	19	15	3	44
No. (%) of clustered isolates	211 (86.8%)	47 (42.0%)	34 (38.6%)	10 (41.7%)	264 (74.4%)
Maximum no. of isolates in a cluster	77	5	5	5	82
No. (%) of cluster further differentiated by RFLP	25 (92.6%)	15 (78.9%)	12 (80.0%)	2 (66.7%)	39 (88.6%)
HGI	0.8827	0.9929	0.9932	0.9493	0.9376
IS*6110* RFLP					
Total type pattern	203	99	83	16	302
No. of isolates having unique type (%)	178 (73.3%)	91 (81.25%)	78 (88.6%)	13 (54.2%)	269 (75.8%)
No. of clusters	25	8	5	3	34
No. of clustered isolates (%)	65 (26.7%)	21 (18.75%)	10 (11.4%)	11 (45.8%)	86 (24.2%)
Maximum no. of isolates in a cluster	5	6	2	6	6
No. of cluster further differentiated by MIRU (%)	10 (40%)	4 (50%)	2 (40%)	2 (66.7%)	14 (41.2%)
HGI	0.9979	0.9961	0.9987	0.9312	0.9986

[a] Non-Beijing strains are further divided into isolates with five or more IS*6110* bands and isolates with fewer than five bands.

total of 135 types (HGI 0.9376) (Table 2). Among the Beijing family isolates, MIRU typing detected 60 types (HGI 0.8837), while in the non-Beijing family group, it was able to detect 83 types (HGI 0.9929), with a breakdown of 68 types (HGI 0.9932) for isolates having five or more IS*6110* bands and 17 types (HGI 0.9493) for those with less than five IS*6110* bands.

When individual MIRU loci were compared, all MIRU loci appeared to perform better in differentiating non-Beijing isolates (i.e., a higher HGI) than in differentiating Beijing isolates (Table 3). All Beijing strains studied showed no variation for loci MIRU-2 and MIRU-24, with a tandem-repeat number of 2 and 1 respectively. None of the MIRU had HGI of more than 0.5 for Beijing strains, indicating a poor discriminating power in this family. A total of 27 clusters were found among the Beijing strains. The largest cluster was (2233-2517-3533), with 77 members, followed by (2223-2517-3543) and (2233-2517-3532), with 23 and 12 members, respectively (Table 4).

TABLE 3. Comparison of HGI values of various MIRUs loci for typing of *M. tuberculosis* strains

Marker	HGI value for:			
	Beijing genotype (n = 243)	Non-Beijing genotype[a]		
		All strains (n = 112)	>5 bands (n = 88)	≤5 bands (n = 24)
MIRU 2	0.0000	0.1182	0.1481	0.0000
MIRU 4	0.0723	0.4903	0.3393	0.7899
MIRU 10	0.2817	0.7734	0.7806	0.6304
MIRU 16	0.0801	0.4410	0.4882	0.2355
MIRU 20	0.0082	0.0861	0.1081	0.0000
MIRU 23	0.1102	0.4202	0.4545	0.2899
MIRU 24	0.0000	0.2106	0.1857	0.3043
MIRU 26	0.3030	0.7851	0.7837	0.6884
MIRU 27	0.1754	0.3470	0.2061	0.5543
MIRU 31	0.1557	0.6662	0.7011	0.4674
MIRU 39	0.3564	0.5481	0.5452	0.4203
MIRU 40	0.4091	0.6977	0.7098	0.5833
All MIRUs	0.8827	0.9929	0.9932	0.9493
IS*6110* RFLP	0.9979	0.9961	0.9987	0.9312

[a] Non-Beijing strains are further divided into isolates with five or more IS*6110* bands and isolates with fewer than five bands.

Cluster analysis was performed using MIRU data for all isolates. A large group consisting of all Beijing genotype isolates (243 isolates) and a small number of non-Beijing genotype isolates (37 isolates) was found. This group was separated from other non-Beijing-isolate group at categorical coefficient of approximately 60% (Fig. 1). A more diverse genotyping pattern was observed among non-Beijing strains, which segregated into four distinct groups, separating at categorical coefficients of 43, 47, and 60%. Moreover, within the mixed group of predominantly Beijing and non-Beijing strains, only 15 non-Beijing isolates shared MIRU patterns with the Beijing group, and these occurred within nine different patterns. When considering all 60 MIRU patterns obtained in the Beijing family (Table 2), only 9 such patterns were shared with the non-Beijing members.

IS*6110* RFLP typing. IS*6110* RFLP typing of the 355 *M. tuberculosis* isolates resulted in 302 RFLP patterns (HGI 0.9986) (Table 2). Among the Beijing family isolates, RFLP detected 203 patterns (HGI 0.9979), while in the non-Beijing family group, it was able to detect 99 patterns (HGI 0.9961). Further breakdown showed that there were 83 types (HGI 0.9987) for isolates having five or more IS*6110* bands and 16 types (HGI 0.9312) for those with fewer than five IS*6110* bands (Table 2). Unlike for MIRU typing, the discriminating power, as measured by the HGI value, in Beijing family (0.9979) and

TABLE 4. MIRU clusters for the Beijing genotype

MIRU pattern[a]	No. of isolates in cluster		
	MDR	non-MDR	Total
2233-2517-3533	22	55	77
2223-2517-3543	7	16	23
2233-2517-3532	5	7	12
2233-2517-1531	1	7	8
2223-2517-3533	2	7	9
2233-2517-3433	5	2	7
2233-2617-1531	5	1	6
2233-2517-3534	1	5	6
2233-2518-3533	0	6	6
2233-2517-3553	1	4	5
2233-2517-3543	1	4	5
2234-2517-3532	3	2	5
2233-2517-3523	0	4	4
2133-2517-3533	3	1	4
2233-2515-3533	1	3	4
2233-2516-3533	0	4	4
2233-2516-3534	0	3	3
2033-2517-3534	0	3	3
2233-2517-3633	1	2	3
2232-2517-3533	0	3	3
2033-2517-3533	0	2	2
2233-2517-3542	1	1	2
2233-2518-3553	1	1	2
2223-2517-3532	0	2	2
2233-2516-3733	1	1	2
2233-2217-4433	0	2	2
2223-2517-3544	0	2	2

[a] Order of MIRU loci: 2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39, 40.

non-Beijing strains (0.9961) was very similar. Among the non-Beijing group, however, isolates with fewer than five IS*6110* bands had a considerably smaller HGI value (0.9312) than the overall HGI value (0.9986) for all strains.

Comparison of MIRU and IS*6110* RFLP typing. Considering all isolates, 39 of the 44 MIRU clusters could be further subdivided by IS*6110* RFLP typing. Within these MIRU clusters, 25 (92.6%) of 27 Beijing types and 15 (78.9%) of 19 non-Beijing types produced diverse RFLP patterns (Table 2). On the other hand, for the 25 RFLP clusters found in the Beijing family, members from 10 clusters (40.0%) could be further differentiated by MIRU. Four (50.0%) of eight non-Beijing RFLP clusters were further differentiated by MIRU (Table 2). For the Beijing family, the addition of RFLP to MIRU offered a higher discrimination ability (92.6%) than did the addition of MIRU to RFLP (40.0%).

Comparison of MDR and non-MDR strains. The HGI values for both MIRU and IS*6110* RFLP were higher for the non-MDR than the MDR strains in our collection. This is irrespective of whether they belonged to the Beijing or non-Beijing family (Table 5). Also, it appeared that the MDR strains circulating in the community were more clonal than the non-MDR ones.

DISCUSSION

DNA fingerprinting is an important tool for tracking the spread and studying the global diversity of *M. tuberculosis*. With the increasing recognition of the importance of the Beijing genotype family in the worldwide TB epidemic (2, 10), the availability of a suitable typing method for this family, which can be used for large-scale typing, became a matter of utmost importance in the global control of TB. Due to its high resolution, simplicity, sensitivity, high reproducibility, and easy interlaboratory comparison (15, 18, 25), the 12-locus MIRU typing method has been found to be highly suitable for global epidemiological surveillance of TB. MIRU typing was even found to produce more distinct patterns than IS*6110* RFLP and spoligotyping (1, 4). In 2002, following the adoption of an agreed International Standard Protocol, a consensus was reached in the European Union Concerted Action meeting, New Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis (Cascais, Portugal, 2002), that MIRU-based typing methods would supersede IS*6110*-based methods in the near future, following adoption of an agreed International Standard Protocol. However, only limited information about the use of MIRU typing was available for the differentiation of the increasingly important Beijing family of *M. tuberculosis* strains.

In the present study, 355 drug-resistant *M. tuberculosis* isolates (68.5% of which were members of the Beijing family) were analyzed by the 12-locus MIRU typing method and the results were compared to those obtained with IS*6110* RFLP. Only for non-Beijing *M. tuberculosis* isolates, MIRU typing (HGI = 0.9929) (compared with HGI = 0.9961 for IS*6110* RFLP) showed similar promising results to those found previously elsewhere (1, 4, 25). For isolates of the Beijing family, however, MIRU typing (HGI = 0.8827) was unable to produce sufficient resolution to facilitate strain differentiation compared with IS*6110* RFLP (HGI = 0.9979); this was apparent regardless of the MDR status of the isolates. This discriminatory power of MIRU was even lower than that obtained for IS*6110* RFLP typing for isolates with fewer than five IS*6110* bands (HGI = 0.9312), since these strains were well known to require additional markers for optimal typing (8, 30).

Since all strains tested in this study were only local isolates, sampling bias might explain the apparent clonal appearance of Beijing family strains in MIRU typing. The method of strain selection could have introduced a bias since all MDR strains were chosen and only a random sample of non-MDR isolates was used. There could thus be a greater tendency to recover any clusters of MDR isolates. Comparable resolution of the non-Beijing genotype MIRU typing with that obtained by other investigators (1, 4, 25), however, and the high resolution of RFLP typing excluded this possibility. In the final analysis, correlation of discriminatory power must be done as far as possible with actual transmission patterns and epidemiological studies obtained from using conventional methods of contact tracing.

When fingerprints of MDR and non-MDR strains were compared by either the RFLP or MIRU method, the MDR strains appeared to be more clonal than the non-MDR strains (Table 5). This was not unexpected, since the absolute number of MDR strains and thus the number of clones circulating in a community should be much smaller than those for the non-MDR ones. Apart from the fact that MDR strains were slightly more clonal than the non-MDR strains in this study, there was virtually no difference in the distribution of typing patterns between MDR and non-MDR strains. This explains why sub-

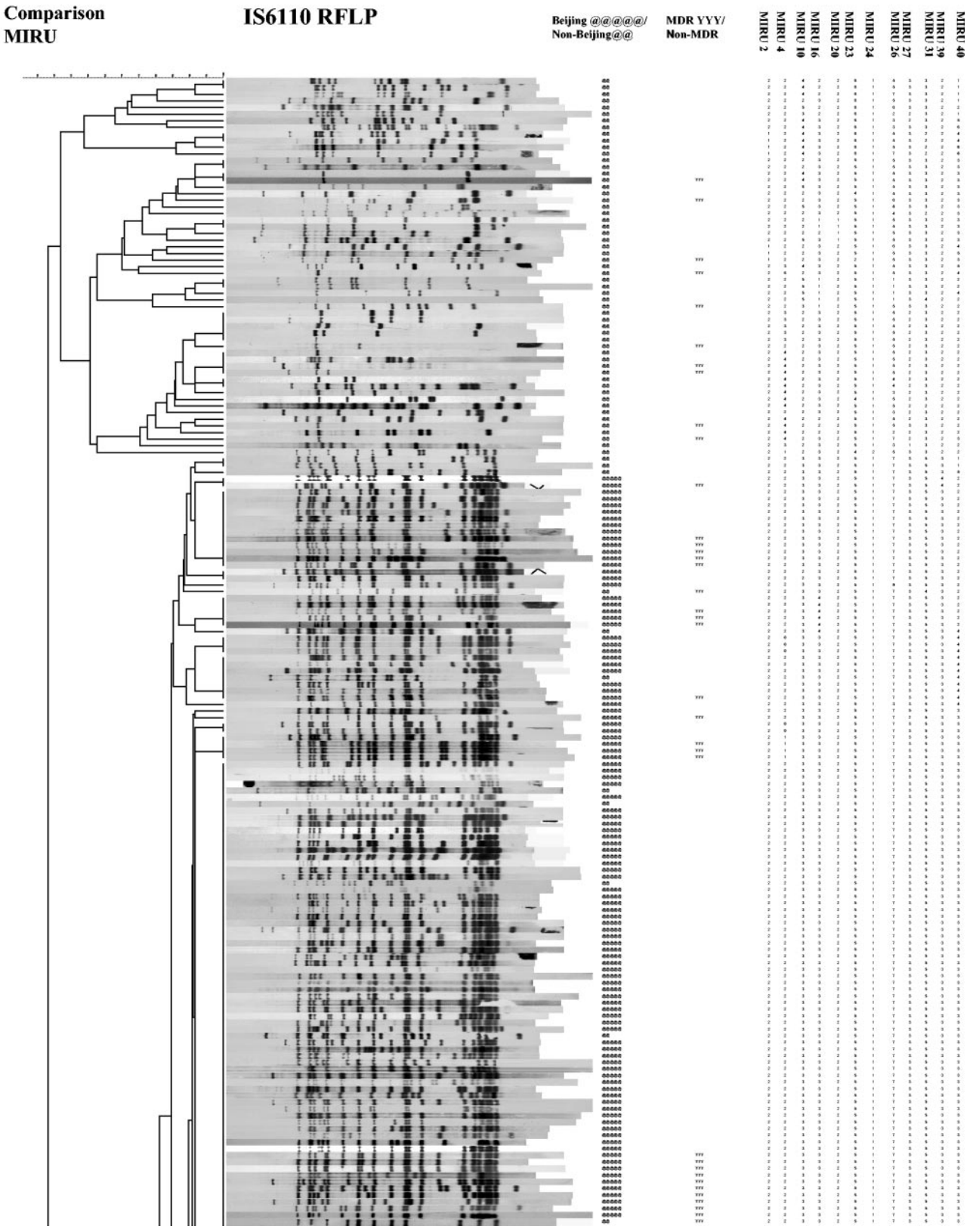

FIG. 1. Dendrogram showing clustering of VNTR-MIRU typing.

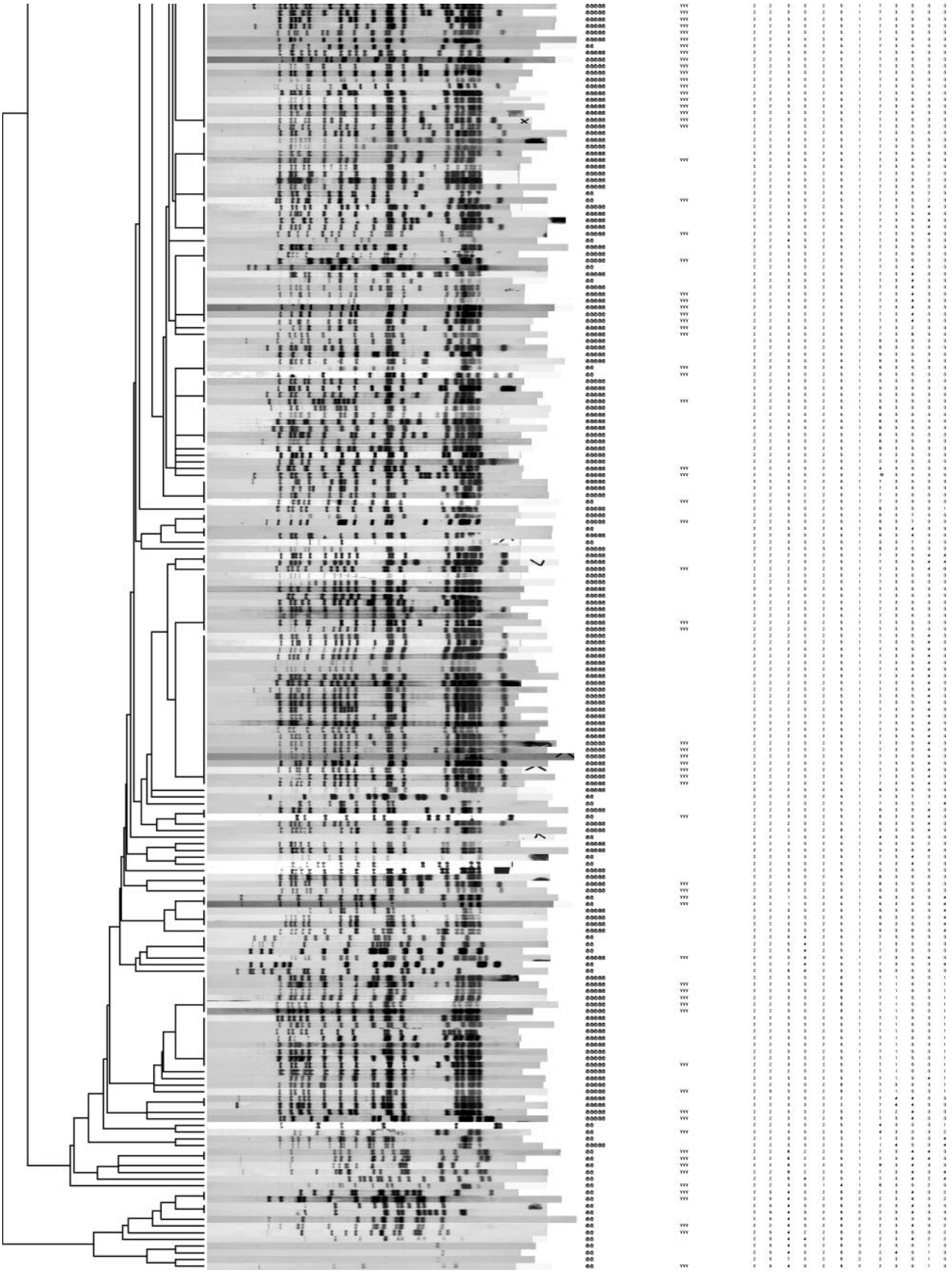

FIG. 1—*Continued.*

TABLE 5. Comparison of strain differentiation between MDR and non-MDR strains

Strain-typing markers	Beijing genotype (n = 243)	Non-Beijing genotype[a]	
		≥5 bands (n = 88)	<5 bands (n = 24)
MDR strains			
No. of isolates	72	22	8
HGI (12 MIRUs)	0.8869	0.9827	0.9643
HGI (IS*6110* RFLP)	0.9902	0.9913	0.8929
Non-MDR strains			
No. of isolates	171	66	16
HGI (12 MIRUs)	0.8806	0.9921	0.9167
HGI (IS*6110* RFLP)	0.9981	0.9986	0.95

[a] Non-Beijing strains are further divided into isolates with five or more IS*6110* bands and isolates with fewer than five bands.

sequent analyses were performed regardless of the MDR status of strains.

Members of the Beijing family constitute a homogeneous group of mycobacteria, as demonstrated by their highly similar RFLP patterns and unique family-specific spoligotyping patterns (27). This homogeneity was also observed in MIRU typing. All HGI values of individual MIRU loci of the Beijing family were less than 0.5, and no variation was observed even for MIRU-2 and MIRU-24 (Table 3). On the other hand, (42%) of 12 MIRU loci from the non-Beijing *M. tuberculosis* strains gave a score of 0.5, with a maximum value of 0.7851 (MIRU-26) followed by 0.7734 (MIRU-10) (Table 3). This observation implied a relatively greater diversity of genetic makeup of the non-Beijing strains. Moreover, a dendrogram constructed with the MIRU data showed a clear segregation between the Beijing and non-Beijing genotypes (Fig. 1). This supported the potential use of this method to track the spread and analyze the global genetic diversity of MTB strains at different levels of evolutionary divergence (25).

Despite the finding that MIRU typing did not provide sufficient resolution for the subtyping of Beijing genotype *M. tuberculosis* strains compared with IS*6110* RFLP, the advantages of VNTR typing over RFLP are obvious; these include the facts that it is PCR based and easy to perform, and gives data that are easily interpretable and exchangeable between laboratories. Therefore, further efforts should be expanded to improve its resolution for the Beijing family genotype. Apart from MIRU typing, other investigators have used exact tandem repeat loci A to E (7) and various other loci identified at the Queens University of Belfast (21) for the typing of *M. tuberculosis*. Recently, Spurgiesz et al. (23) subjected 34 Beijing family strains to VNTR typing with nine VNTR loci and produced seven VNTR types. Investigations aimed at using various VNTR loci against Beijing strains should be conducted to improve the resolution of this method to a level comparable to that of the RFLP method. Moreover, our study showed that MIRU typing could still possess additional differentiation power in 10 (40%) of 25 RFLP clusters, even though members of Beijing family was so clonal (Table 2). Since additional contact tracing, which is both time-consuming and labor-intensive, would be needed for RFLP cluster cases, the use of MIRU typing for RFLP cluster group would provide additional information that would minimize unnecessary contact tracing efforts.

With the promising results obtained from MIRU typing of *M. tuberculosis*, standardization of laboratory methods using MIRU typing is of paramount importance in the construction of digital global databases of *M. tuberculosis* molecular epidemiology (15, 18, 25). However, using the present set of MIRUs, its limitations for typing the Beijing family strains must be seriously considered. In theory, we can increase the number of loci to improve the resolution. However, because most Beijing strains are prevalent in many of the developing areas of Asia where the TB disease burden is high and resources are definitely limited, only an affordable number of loci should be used for typing. This means that, efforts must be expanded in improving the methodology to cut down costs. A plausible approach would involve doing multiplex PCRs to cut down PCR tube numbers or discovering and incorporating a few highly differentiating loci. This must be done before this VNTR typing method can be validated and made applicable to parts of the world with a high burden of infection by Beijing family strains.

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